PCT/EP2004/012572

REC'D 09 DEC 2004
WIPO PCT

PA 1236766

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

October 18, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/518,073*
FILING DATE: *November 07, 2003*

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

N. WOODSON
Certifying Officer

Use of organic compounds

The invention relates to medical uses of fibroblast growth factor 23 (FGF-23), FGF-23 fragments, FGF-23 C-terminal polypeptides, FGF-23 homologs and/or FGF-23 variants, in particular for the manufacture of a medicament for the treatment of diseases associated with deregulated angiogenesis or cell proliferative disorders.

Background

Fibroblast growth factors (FGFs) make up a large family of polypeptide growth factors that are found in organisms ranging from nematodes to humans. During embryonic development, FGFs have diverse roles in regulating cell proliferation, migration and differentiation. In the adult organism, FGFs are homeostatic factors and function in tissue repair and response to injury. Inappropriate expression of some FGFs can contribute to the pathogenesis of cancer.

Mouse FGF-23 has been identified by homology search in the GenBank Nucleotide Sequence Database with amino acid sequence of mouse FGF-15. Mouse FGF-23 and human FGF-23 are highly identical (~72% amino acid identity). Both, mouse and human FGF-23, cDNAs encode a protein of 251 amino acids, having a hydrophobic amino terminus (~24 amino acids) typical for secreted proteins, and a unique C-terminus having no homology to other FGF family members. In the mouse, FGF-23 mRNA is expressed in the brain, preferentially in the ventrolateral thalamic nucleus, and in the thymus at low levels.

Overexpression of FGF-23 or expression of mutated FGF-23 has been demonstrated to be associated with several pathological findings:

Recombinant FGF-23 induces hypophosphatemia *in vivo* as a result of urinary phosphate wasting (Shimada T., et al., Proc. Natl. Acad. Sci. U. S. A. 98: 6500-6505 (2001)).

FGF-23 overexpression has been observed in tumors that are responsible for oncogenic osteomalacia (OOM) (White K.E., et al., J. Clin. Endocrinol. Metab. 86: 497-500 (2001)).

Autosomal dominant hypophosphatemic rickets (ADHR) has been shown to be associated with mutations of FGF-23 within the 176-RXXXR-179 cleavage site, preventing degradation of FGF-23 (The ADHR Consortium, Nat. Genet. 26: 345-348 (2000)).

While OOM and ADHR have been demonstrated to be associated with FGF-23, a further disorder, X-linked hypophosphatemia (XLH), which is phenotypically similar to OOM and ADHR, has been shown to result from mutations in the PHEX gene. PHEX encodes a membrane-bound endopeptidase (The HYP Consortium, Nat. Genet. 11: 130–136 (1995)) and FGF-23 is hypothesized to be a PHEX substrate, while FGF-23 ADHR mutant (FGF-23(R179Q) being undegradeable by PHEX.

Each of the above described syndromes is characterized by hypophosphatemia, decreased renal phosphate reabsorption, normal or low serum calcitriol concentrations, normal serum concentrations of calcium and parathyroid hormone, and defective skeletal mineralization (Quarles L. D. and Drezner M. K., J. Clin. Endocrinol. Metab. 86: 494–496 (2001)).

Because both the overproduction and missense mutations of FGF-23 cause hypophosphatemia with renal phosphate wasting, it is concluded that FGF-23 is at least one of the causative factors of OOM and is an important regulator of phosphate and bone metabolism (Shimada T., Proc. Natl. Acad. Sci. USA 98: 6500–6505(2001)). However, the molecular targets of FGF-23 or of FGF-23 proteolytic cleavage products are so far unknown, as is the mechanism of how FGF-23 or FGF-23-derived proteins or peptides cause renal and skeletal abnormalities (Quarles L. D., Am. J. Physiol. Endocrinol. Metab. 285: E1 – 9 (2003)).

Summary of the Invention

Surprisingly, it has now been found that polypeptides relating to FGF-23 affect key genes controlling cellular differentiation and proliferation, as well as angiogenesis.

The present invention relates to the use of a polypeptide for the manufacture of a medicament for use in the treatment of a disease associated with deregulated angiogenesis, wherein the polypeptide is selected from the groups consisting of a) fibroblast growth factor 23 (FGF-23) (SEQ. ID No: 1) or a fragment of FGF-23; b) a bioactive polypeptide having a percentage of identity of at least 50% with the amino acid sequence of any one of the polypeptides of (a); or c) a bioactive variant of any one of the polypeptides of (a) or (b).

In a further aspect, the present invention relates to the use of a polypeptide as defined above for the manufacture of a medicament for use in the treatment of a cell proliferative disorder.

In a further aspect, the present invention relates to a method for the treatment of a disease associated with deregulated angiogenesis or of a cell proliferative disorder comprising administering an effective amount of a polypeptide as defined above to a mammal including a human suffering from the disease or disorder.

In another aspect, the present invention relates to a pharmaceutical composition for use in a disease associated with deregulated angiogenesis or a cell proliferative disorder comprising a polypeptide as defined above and a pharmaceutically-acceptable carrier.

Brief description of the Figures

Figure 1 is a box plot of the proliferation scores (Units) relating to vascular changes on retinal flatmounts of animals treated with FGF-23 C-terminal polypeptide (FGF23CTP; (right eye; column 1) and PBS (left eye; column 1).

Detailed description of the invention

The present invention provides for the use of a polypeptide for the manufacture of a medicament for use in the treatment of a disease associated with deregulated angiogenesis, wherein the polypeptide is selected from the groups consisting of a) fibroblast growth factor 23 (FGF-23) (SEQ. ID No: 1) or a fragment of FGF-23; b) a bioactive polypeptide having a percentage of identity of at least 50% with the amino acid sequence of any one of the polypeptides of (a); or c) a bioactive variant of any one of the polypeptides of (a) or (b).

According to another aspect, the present invention provides for the use of a polypeptide as defined above for the manufacture of a medicament for use in the treatment of a cell proliferative disorder.

SEQ. ID No: 1 (Human FGF-23 amino acid sequence)

```
        10         20         30         40         50         60
         |          |          |          |          |          |
MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH

        70         80         90        100        110        120
         |          |          |          |          |          |
VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL
```

```
       130        140        150        160        170        180
         |          |          |          |          |          |
ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTRS

       190        200        210        220        230        240
         |          |          |          |          |          |
AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG

       250
         |
PEGCRPFAKF I
```

The term "polypeptide" as used herein, refers to a protein, peptide, oligopeptide or synthetic oligopeptide. These terms are intended to be used interchangeably. Any one of said terms refers to a chain of two or more amino acids which are linked together with peptide or amide bonds, regardless of post-translational modification such as glycosylation or phosphorylation. The polypeptides may also comprise more than one subunit, where each subunit is encoded by a separate DNA sequence.

The term "bioactive", as used herein, refers to a molecule that elicits or affects a biological event. Such biological event may for example be related to a disease associated with deregulated angiogenesis or to a cell proliferative disorder.

A "bioactive polypeptide" of the invention includes FGF-23, fragments of FGF-23 such as fragments derived from the C-terminus of FGF-23 . Also included are homologs which have an amino acid sequence having a percentage of identity of at least 50% to FGF-23 or fragments thereof and variants of FGF-23 or of FGF-23 fragments. The polypeptide according to the invention may comprise FGF-23 having the amino acid sequence of SEQ ID NO: 1. A fragment of FGF-23 may comprise at least 10 amino acids, preferably at least 15, 20, 25 or 30 amino acids. More preferably a fragment of FGF-23 may comprise at least 50, 60, or 70 amino acids. Most preferably a fragment of FGF-23 comprises 75 amino acids. Alternatively, a fragment of FGF-23 may comprise at least 80 or 100 amino acids, and most preferred at least 120 or 150 amino acids. In particular, the fragment may comprise at least 180 amino acids, such as e.g. 200 amino acids.

Such polypeptide may also be a proteolytic cleavage product of FGF-23 generated by proteases such as a membrane-bound endopeptidase including PHEX. A polypeptide according to the invention may comprise a C-terminal fragment of FGF-23. Such C-terminal fragment may comprise at least 15 amino acids of the C-terminus of FGF-23, preferably at least 25, at least 35 or 45, more preferably at least 55 or at least 65, most preferred at least

polypeptide comparisons. As used herein, an "optimal alignment" of sequences being compared is one that maximizes matches between subunits and minimizes the number of gaps employed in constructing an alignment. Percent identities may be determined with commercially available implementations of algorithms described by Needleman and Wunsch, J. Mol. Biol. 48: 443-453 (1970)("GAP" program of Wisconsin Sequence Analysis Package, Genetics Computer Group, Madison, WI). Other software packages in the art for constructing alignments and calculating percentage identity or other measures of similarity include the "BestFit" program, based on the algorithm of Smith and Waterman, Advances in Applied Mathematics 2: 482-489 (1981) (Wisconsin Sequence Analysis Package, Genetics Computer Group, Madison, WI). The percentage of identity may also be generated by WU-BLAST-2 (Altschul et al., Methods in Enzymology 266: 460-480 (1996)). WU-BLAST-2 used several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11. A % amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues in the aligned region. For example, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to five percent of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to five percent of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence of in one or more contiguous groups with in the references sequence. It is understood that in making comparisons with reference sequences of the invention that candidate sequence may be a component or segment of a larger polypeptide or polynucleotide and that such comparisons for the purpose computing percentage identity is to be carried out with respect to the relevant component or segment.

A polypeptide of the invention also includes a polypeptide fragment of a polypeptide of the invention. Such polypeptide fragment is meant to be a polypeptide having an amino acid sequence that entirely is the same in part, but not in all, of the amino acid sequence of a polypeptide of the invention. Such polypeptide fragment may be "free-standing," or may be part of a larger polypeptide of which such polypeptide fragment forms a part or region, most preferably as a single continuous region. Preferably such polypeptide or polypeptide fragment retains the biological activity of the corresponding polypeptide of the invention.

The invention also includes functionally preserved variants of the polypeptides or polypeptide fragments described herein. Such variants may be made using methods standard in the art, for example, by conservative amino acid substitutions. Typically such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5 to 10, 1 to 5, or 2 amino acids are substituted, deleted or added, in any combination.

In various other embodiments, the polypeptide (fragment) or polypeptide variant may be linear or branched, it may comprise modified amino acids, it may be interrupted by non-amino acids, and/or it may be assembled into a complex of more than one polypeptide chain. As is well understood in the art, a polypeptide may be modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. In some embodiments, polypeptides or polypeptide fragments contain one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

A polypeptide or a polypeptide fragment of the invention includes isolated naturally occurring polypeptides. Preferably, such a naturally occurring polypeptide has a frequency in a selected population of at least five percent, and most preferably, of at least ten percent. The selected population may be any recognized population of study in the field of population genetics. Preferably, the selected population is Caucasian, Negroid, or Asian. More preferably, the selected population is French, German, English, Spanish, Swiss, Japanese, Chinese, Korean, Singaporean of Chinese ancestry, Icelandic, North American, Israeli, Arab, Turkish, Greek, Italian, Polish, Pacific Islander, or Indian.

A polypeptide (fragment) of the invention may also include recombinantly produced polypeptides, synthetically produced polypeptides and a combination of such polypeptides of the invention, and fragments thereof. Means for preparing such polypeptides are well understood in the art. For instance, a polynucleotide fragment or a polypeptide of the invention can be isolated from body fluids including, but not limited to, serum, urine, and ascites, or synthesized by chemical or biological methods (for example, cell culture, recombinant gene expression). "Isolated", if not otherwise specified herein includes the meaning "separated from coexisting material".

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to the production of polypeptides by recombinant techniques, to expression systems which comprises a nucleic acid or nucleic acids encoding the polypeptides of the present invention, to host cells which are genetically engineered with such expression systems, and to methods to isolate the polypeptides.

Another embodiment provides that a polypeptide of the invention is encoded by a nucleic acid which hybridizes under stringent conditions to SEQ. ID No: 3 or to SEQ. ID No: 4. In some embodiments, the nucleic acid comprises at least 50, at least 75, at least 100, at least 125, or at least 150 nucleotides. Preferably the nucleic acid comprises at least 175 or at least 200 nucleotides. In particular it comprises 225 or 228 nucleotides. The nucleic acid may also comprise at least 300, or at least 400 or 500 nucleotides. Preferably it may comprise at least 600 or at least 700 nucleotides. Most preferably it comprises at least 750 nucleotides. Such nucleic acids may comprise contiguous nucleotides of SEQ ID NO: 3 or 4 or contiguous nucleotides able to hybridize to SEQ ID NO: 3 or 4 under stringent conditions.

SEQ. ID No: 3 (nucleic acid sequence of human FGF-23)

```
atgttggggg cccgcctcag gctctgggtc tgtgccttgt gcagcgtctg cagcatgagc   60
gtcctcagag cctatcccaa tgcctcccca ctgctcggct ccagctgggg tggcctgatc  120
cacctgtaca cagccacagc caggaacagc taccacctgc agatccacaa gaatggccat  180
gtggatggcg caccccatca gaccatctac agtgccctga tgatcagatc agaggatgct  240
ggctttgtgg tgattacagg tgtgatgagc agaagatacc tctgcatgga tttcagaggc  300
aacatttttg gatcacacta tttcgacccg gagaactgca ggttccaaca ccagacgctg  360
gaaaacgggt acgacgtcta ccactctcct cagtatcact tcctggtcag tctgggccgg  420
gcgaagagag ccttcctgcc aggcatgaac ccacccccgt actcccagtt cctgtcccgg  480
aggaacgaga tcccccctaat tcacttcaac acccccatac cacggcggca cacccggagc  540
gccgaggacg actcggagcg ggaccccctg aacgtgctga agccccgggc ccggatgacc  600
ccggccccgg cctcctgttc acaggagctc ccgagcgccg aggacaacag cccgatggcc  660
agtgacccat taggggtggt caggggcggt cgagtgaaca cgcacgctgg gggaacgggc  720
ccggaaggct gccgcccctt cgccaagttc atctag                             756
```

SEQ. ID No: 4 (nucleic acid sequence of FGF23CTP)

```
cacacccgga gcgccgagga cgactcggag cgggaccccc tgaacgtgct gaagccccgg   60
gcccggatga ccccggcccc ggcctcctgt tcacaggagc tcccgagcgc cgaggacaac  120
agcccgatgg ccagtgaccc attaggggtg gtcaggggcg gtcgagtgaa cacgcacgct  180
gggggaacgg gcccggaagg ctgccgcccc ttcgccaagt tcatctag               228
```

The term "nucleic acid" means natural or semi-synthetic or synthetic or modified nucleic acid molecules. It refers to nucleotide sequences, oligonucleotides or polynucleotides including deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA) and/or modified nucleotides. These terms are intended to be used interchangeably. RNA may be in the form of an tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), mRNA (messenger RNA), anti-sense RNA, and ribozymes. DNA may be in form of plasmid DNA, viral DNA, linear DNA, chromosomal or genomic DNA, cDNA, or derivatives of these groups. In addition these DNAs and RNAs may be single, double, triple, or quadruple stranded. The term also includes PNAs (peptide nucleic acids), phosphorothioates, and other variants of the phosphate backbone of native nucleic acids.

"Stringent conditions" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends upon the ability of a denatured nucleic acid to reanneal when complementary strands are present in an environment near but below their melting temperature. The higher the degree of homology between the probe and the hybridizable sequence such as SEQ. ID No: 3 or 4, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. Moreover, stringency is also inversely proportional to salt concentrations. "Stringent conditions" are exemplified by reaction conditions characterized by: (1) low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) the use of a denaturing agent, such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1%

polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C. Alternatively, stringent conditions can be: 50% formamide, 5x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1x SSC containing EDTA at 55°C. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Protocols in Molecular Biology (1995).

Recombinant Manufacture of FGF-23 C-terminal polypeptides

The nucleic acids described herein such as SEQ. ID No: 3 or 4 may be used in recombinant DNA molecules to direct the expression of the corresponding polypeptides in appropriate host cells. Because of the degeneracy in the genetic code, other DNA sequences may encode the equivalent amino acid sequence, and may be used to clone and express FGF-23 or fragments thereof. Codons preferred by a particular host cell may be selected and substituted into the naturally occurring nucleotide sequences, to increase the rate and/or efficiency of expression. The nucleic acid (e.g., cDNA or genomic DNA) encoding the desired FGF-23 or FGF-23 fragments such as FGF23CTP may be inserted into a replicable vector for cloning (amplification of the DNA), and/or for expression.

Expression Systems

The polypeptide can be expressed recombinantly in any of a number of expression systems according to methods known in the art (Ausubel, et al., editors, Current Protocols in Molecular Biology, John Wiley Sons, New York, 1990). Such expression systems include chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids.

The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express

a nucleic acid to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). In general, DNA is inserted into an appropriate restriction endonuclease site using techniques known in the art.

Vector components generally include, but are not limited to, one or more of an origin of replication, one or more marker genes, an enhancer element, a promoter, a signal or secretion sequence, and a transcription termination sequence:

The expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. Such sequences are well known for a variety of bacteria, yeast strains, and viruses.

Preferably, the expression vector contains a marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used. Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients e.g., the D-alanine racemase gene.

Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention. Further, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably, two homologous sequences which flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by insertion of the appropriate homologous sequence in the vector. Constructs for integrating vectors are well known in the art.

An appropriate secretion signal may be incorporated into the desired polypeptide to allow secretion of the polypeptide into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline

phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, the alpha factor leader (including *Saccharomyces* and *Kluyveromyces* a-factor leaders). In mammalian cell expression systems, mammalian signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders may be used to direct secretion of FGF-23 or fragments thereof such as FGF23CTP.

Appropriate host cells include yeast, bacteria, archebacteria, fungi, and insect and animal cells, including mammalian cells, for example primary cells, including but not limited to stem cells. Representative examples of appropriate hosts include bacterial cells, such as *E. coli, Streptococci, Staphylococci, Streptomyces*, and *Bacillus subtilis*; fungal cells, such as *Saccharomyces cerevisiae*, other yeast cells or *Aspergillus*; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing, which cleaves a "prepro" form of the polypeptide, may also be important for correct insertion, folding and/or function.

FGF-23 or fragments thereof such as FGF23CTP may be produced by culturing a host cell transformed with an expression vector containing a nucleic acid encoding an FGF-23 or fragments thereof under the appropriate conditions to induce or cause expression of the protein or polypeptide. In a preferred embodiment of the invention a host cell is provided which is stably or transiently transfected with a nucleic acid of SEQ. ID No: 3 or 4 or transfected with a nucleic acid which hybridizes under stringent conditions to SEQ. ID No: 3 or 4. According to another embodiment of the invention said host cell is cultured to allow expression of FGF-23 or of an FGF-23 fragment, and the polypeptide is isolated from the cell culture.

Transformed host cells include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmic DNA expression vectors, yeast transformed with yeast expression vectors, and insect cells infected with a recombinant insect virus (such as baculovirus), and mammalian expression systems.

The appropriate conditions for expression of FGF-23 or fragments thereof such as FGF23CTP will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculoviral systems used together with insect cells are lytic viruses, and thus harvest time selection can be crucial for product yield.

The desired FGF-23 or FGF-23 fragment may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide. Such heterologous polypeptide is generally placed at the amino- or carboxyl-terminus of FGF-23 or of an FGF-23 fragment and may provide for an epitope tag to which an anti-tag antibody can selectively bind. Accordingly, such epitope tag enables FGF-23 or a fragment thereof to be readily purified by using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Examples of epitope tags are 6xHis or c-myc tag. Alternatively FGF-23 or a fragment thereof may be expressed in the form of e.g. an GST-fusion protein. Appropriate constructs are generally known in the art and are available from commercial suppliers such as Invitrogen (San Diego, Calif.), Stratagene (La Jolla, Calif.), Gibco BRL (Rockville, Md.) or Clontech (Palo Alto, Calif.).

Evaluation of Gene Expression

Gene expression may be evaluated in a sample directly, for example, by standard techniques known to those of skill in the art, e.g., Southern blotting for DNA detection, Northern blotting to determine the transcription of mRNA, dot blotting (DNA or RNA), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be used in assays for detection of nucleic acids, such as specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. Such antibodies may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. Gene expression, alternatively, may be measured by immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to directly evaluate the expression of FGF-23 or of an FGF-23 fragment. Antibodies useful for such immunological assays may be either

monoclonal or polyclonal, and may be prepared against a native sequence FGF-23 or FGF-23 fragments based on the DNA sequences provided herein.

Purification of Expressed Protein

Expressed FGF-23 or an FGF-23 fragment such as FGF23CTP may be purified or isolated after expression, using any of a variety of methods known to those skilled in the art. The appropriate technique will vary depending upon the way of expression of FGF-23 or an FGF-23 fragment. The polypeptide may for example be recovered from culture medium in the form of a secreted potein or from host cell lysates. Cells can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or by use of cell lysing agents, whereas membrane-bound polypeptides may be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by enzymatic cleavage. The appropriate technique for polypeptide purification or isolation will also vary depending upon what other components are present in the sample. The degree of purification necessary will also vary depending on the use of FGF-23 or a fragment thereof. Contaminant components that are removed by isolation or purification are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other solutes. The purification step(s) selected will depend, for example, on the nature of the production process used and the particular FGF-23 or FGF-23 fragment produced.

Ordinarily, isolated FGF-23 or a fragment thereof will be prepared by at least one purification step. Well-known methods for purification include ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, high performance liquid chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, affinity chromatography is employed for purification. For example, the FGF-23 or a fragment thereof such as FGF23CTP may be purified using a standard anti-FGF-23 C-terminal polypeptide antibody column. Ultrafiltration and dialysis techniques, in conjunction with protein concentration, are also useful (see, for example, Scopes, R., Protein Purification, Springer-Verlag, New York, N.Y., 1982). Well-known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification

Labeling of Expressed Polypeptide

The nucleic acids, proteins and antibodies of the invention may be labeled. By labeled herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the compound at any position that does not interfere with the biological activity or characteristic of the compound which is being detected.

Chemical Manufacture of FGF-23 and FGF-23 fragments

Polypeptides or fragments thereof may be produced not only by recombinant methods, but also by using chemical methods well known in the art. Solid phase peptide synthesis may be carried out in a batchwise or continuous flow process which sequentially adds alpha-amino- and side chain-protected amino acid residues to an insoluble polymeric support via a linker group. A linker group such as methylamine-derivatized polyethylene glycol is attached to poly(styrene-co-divinylbenzene) to form the support resin. The amino acid residues are N^{alpha}-protected by acid labile Boc (t-butyloxycarbonyl) or base-labile Fmoc (9-fluorenylmethoxycarbonyl). The carboxyl group of the protected amino acid is coupled to the amine of the linker group to anchor the residue to the solid phase support resin. Trifluoroacetic acid or piperidine are used to remove the protecting group in the case of Boc or Fmoc, respectively. Each additional amino acid is added to the anchored residue using a coupling agent or pre-activated amino acid derivative, and the resin is washed. The full length peptide is synthesized by sequential deprotection, coupling of derivatized amino acids, and washing with dichloromethane and/or N, N-dimethylformamide. The peptide is cleaved between the peptide carboxy terminus and the linker group to yield a peptide acid or amide. (Novabiochem 1997/98 Catalog and Peptide Synthesis Handbook, San Diego Calif. pp. S1-S20). Automated synthesis may also be carried out on machines such as the ABI 431A peptide synthesizer (Applied Biosystems). A polypeptide or a fragment thereof may be purified by preparative high performance liquid chromatography and its composition confirmed by amino acid analysis or by sequencing (Creighton T.E. (1984) Proteins, Structures and Molecular Properties, W H Freeman, New York N.Y.).

Variants of the natural polypeptide may be desirable in a variety of circumstances. For example, undesirable side effects might be reduced by certain variants, particularly if the side effect activity is associated with a different part of the polypeptide from that of the desired activity. In some expression systems, the native polypeptide may be susceptible to degradation by proteases. In such cases, selected substitutions and/or deletions of amino acids which change the susceptible sequences can significantly enhance yields. Variants may also increase yields in purification procedures and/or increase shelf lives of proteins by eliminating amino acids susceptible to oxidation, acylation, alkylation, or other chemical modifications. Preferably, such variants include alterations that are conformationally neutral, i.e. they are designed to produce minimal changes in the tertiary structure of the variant polypeptides as compared to the native polypeptide, and (ii) antigenically neutral, i.e. they are designed to produce minimal changes in the antigenic determinants of the variant polypeptides as compared to the native polypeptide.

The aforementioned polypeptides may according to the invention be used for the manufacture of a medicament for use in the treatment of a disease associated with deregulated angiogenesis or a cell proliferative disorder.

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

A "disorder" or a "disease" is any condition that would benefit from treatment with FGF-23 or a fragment of FGF-23 as defined above and further below. This includes both chronic and acute disorders, as well as those pathological conditions which predispose to the disorder or disease in question. Non-limiting examples of disorders or diseases to be treated herein include any condition which results from deregulated angiogenesis or from deregulated cell proliferation. Examples of diseases associated with deregulated angiogenesis include: ocular neovascularisation, such as retinopathies (including diabetic retinopathy), age-related macular degeneration, psoriasis, haemangioblastoma, haemangioma, arteriosclerosis, inflammatory diseases, such as rheumatoid or rheumatic inflammatory diseases, especially arthritis, such as rheumatoid arthritis, or other chronic inflammatory disorders, such as chronic asthma, arterial or post-transplantational atherosclerosis, endometriosis, and especially neoplastic diseases, for example so-called solid tumors and liquid tumors (such as leukemias).

A preferred example of a diseases associated with deregulated angiogenesis is selected from the group of retinopathies, age-related macular degeneration, haemangioblastoma, haemangioma, and tumors. A particularly preferred example of a diseases associated with deregulated angiogenesis is retinopathy.

Examples of cell proliferative disorders include: chronic or acute renal diseases, e.g. diabetic nephropathy, malignant nephrosclerosis, thrombic microangiopathy syndromes or transplant rejection, or especially inflammatory renal disease, such as glomeruloneephritis, especially mesangioproliferative glomerulonephritis, haemolytic-uraemic syndrome, diabetic nephropathy, hypertensive nephrosclerosis, atheroma, arterial restinosis, actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and autoimmune diseases, acute inflammation, fibric disorders (e.g. hepatic cirrhosis), diabetes, endometriosis, chronic asthma, neurodegenerative disorders and especially neoplastic diseases such as adenocarcinoma, gliomas, leukemia, lymphoma, melanoma, myeloma, sarcoma, Kaposi's sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, colon, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung (especially small-cell lung cancer), muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

A preferred example of a cell proliferative disorder is selected from the group of chronic or acute renal diseases, arteriosclerosis, atherosclerosis, psoriasis, endometriosis, diabetes, chronic asthma and cancer. A particularly preferred example of a cell proliferative disorder is cancer.

Another aspect of the invention relates to a method for the treatment of a disease associated with deregulated angiogenesis which comprises administering an effective amount of a polypeptide to a mammal including a human suffering from the disease, wherein the polypeptide is selected from the groups consisting of a) FGF-23 (SEQ. ID No: 1) or a fragment of FGF-23; b) a bioactive polypeptide having a percentage of identity of at least 50% with the amino acid sequence of any one of the polypeptides of (a); or c) a bioactive variant of any one of the polypeptides of (a) or (b). Accordingly, a polypeptide as described above may be administered.

Another aspect of the invention relates to a method for the treatment of a cell proliferative disorder comprising administering an effective amount of FGF-23 or an FGF-23

fragment such as FGF23CTP as described above to a mammal including a human suffering from the disorder.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and from animals, and zoo, sports, or pet animals, such as dogs, horses, cats, sheep, pigs, cattle, etc. Preferably, the mammal is human.

Another aspect of the invention provides a pharmaceutical composition for use in a disease associated with deregulated angiogenesis or a proliferative disorder comprising FGF-23 or a fragment thereof according to the invention as described above and a pharmaceutically-acceptable carrier. The composition of the invention is administered in effective amounts.

The pharmaceutical composition may be used in the foregoing methods of treatment. Such compositions are preferably sterile and contain an effective amount of FGF-23 or an FGF-23 fragment such as FGF23CTP or a nucleic acid encoding the polypeptide for inducing the desired response in a unit of weight or volume suitable for administration to a patient.

An "effective amount" of FGF-23 or fragment thereof, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results including clinical results such as inhibiting premature or diabetic retinopathy, inhibiting angiogenesis, shrinking the size of the tumor, retardation of cancerous cell growth, decreasing one or more symptoms resulting from the disease or disorder, increasing the quality of life of those suffering from the disease or disorder, decreasing the dose of other medications required to treat the disease or disorder, enhancing effect of another medication, delaying the progression of the disease or disorder, and/or prolonging survival of patients, either directly or indirectly.

Such amounts will also depend on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

An effective amount can be administered in one or more administrations and may or may not be achieved in conjunction with another drug, compound, or pharmaceutical

composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

An effective amount of FGF-23 or an FGF-23 fragment or the pharmaceutical composition comprising the polypeptide of the invention, alone or in conjunction with another drug, compound, or pharmaceutical composition can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, topical or transdermal.

When administered, the pharmaceutical composition of the present invention is administered in pharmaceutically acceptable preparations. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a mammals including humans. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application.

The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain pharmaceutically acceptable concentrations of salts, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents, such as chemotherapeutic agents.

When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention.

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

The doses of polypeptide or nucleic acid encoding said polypeptide administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors include the

desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous or non-aqueous preparation of a polypeptide or nucleic acid encoding the polypeptide, which is preferably isotonic with the blood of the recipient. This preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

Experiment 1

Methods

FGF23CTP is administered subcutaneously to cynomolgus monkeys for two weeks at a dose of 100 microg/day. At the end of the treatment period samples from all organs are subjected to snap freezing at necropsy and are analyzed with GeneChip® expression profiling.

Total RNA is extracted from these frozen tissues using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. Total RNA is quantified by the absorbance at $\lambda = 260$ nm (A_{260nm}), and the purity is estimated by the ratio A_{260nm}/A_{280nm}. Integrity is checked by denaturing gel electrophoresis. RNA is stored at -80°C until analysis.

Good quality total RNA is used to synthesize double-stranded cDNA using the Superscript Choice System (Life Technologies). The cDNA is then *in vitro* transcribed (MEGAscript™ T7 Kit, Ambion) to form biotin labeled cRNA. Next, 12 to 15 µg of labeled cRNA is hybridized to the Affymetrix Human U95A Version 2 expression probe arrays for 16 hours at 45°C. Arrays are then washed according to the EukGE-WS2 protocol (Affymetrix), and stained with 10 µg/ml of streptavidin-phycoerythrin conjugate (Molecular Probes). The signal is antibody-amplified with 2 mg/ml acetylated BSA (Life Technologies), 100 mM MES, 1 M [Na+], 0.05 % Tween 20, 0.005 % Antiofoam (Sigma), 0.1mg/ml goat IgG and 0.5 mg/ml biotinylated antibody and re-stained with the streptavidin solution. After washing, the arrays are scanned twice with the Gene Array® scanner (Affymetrix).

The expression level is estimated by averaging the differences in signal intensity measured by oligonucleotide pairs of a given probe (AvgDiff value). The image acquisition and numerical translation software used for this study is the Affymetrix Microarray Suite version 4 (MAS4).

To identify genes that are impacted by treatment, the dataset is initially filtered to exclude in a first wave of analysis genes whose values are systematically in the lower expression ranges where the experimental noise is high (at least an AvgDiff value of 80 in a number of experiments corresponding to the smallest number of replicas of any experimental point). In a second round of selection a threshold t-test p-value (0.05) identifies genes with different values between treated and non-treated based on a two component error model (Global Error Model) and, where possible, with a stepdown correction for multi-hypothesis testing (Benjamini and Hochberg false discovery rate).

The selected genelists are then compared with established genelists for pathways and cellular components using Fisher's exact test. Venn diagrams are used to identify the gene changes that are in common between the different organs. Expression profiles of highly relevant genes are used to find genes with correlated changes at individual experimental points, using several distance metrics (standard, Pearson).

The decision to consider a specific gene relevant is based on a conjunction of numerical changes identified by exploratory filtering and statistical algorithms as described above and the relationship to other modulated genes that point to a common biological theme.

Results

A cross comparison of organs analyzed reveals that FGF23CTP affects the same or closely related pathways and provokes similar cellular effects. No routine clinical or biochemical changes are observed in the treated animals. Surprisingly, no effect on phosphate metabolism is observed.

Tables 1 to 4 show that at the RNA level FGF23CTP affects key genes controlling cellular differentiation and proliferation, especially growth factors and growth factor receptors (Table 1). Genes critically involved in angiogenesis are found to be altered in several organs upon treatment with FGF23CTP (Table 2). There is also a multi-organ effect on transcripts for components of the retinoblastoma cycling control checkpoint (Table 3). The rostral hypothalamus shows the most pronounced changes especially on transcripts corresponding to proteins involved in transport and cytoarchitecture (Table 4).

In particular, FGF23CTP affects several molecules that have been described to play a role in the pathogenesis of malignant proliferation of glial cells and precurors: Epidermal growth factor (EGF) (Hoi Sang U., et al., J. Neurosurg. 82: 841-846 (1995); Wu C.J., et al., Oncogene 19: 3999-4010 (2000)), Bax (Streffer J.R., et al., J. Neurooncol. 56: 43-49 (2002); Martin S., et al., J. Neurooncol. 52: 129-139 (2001)), connexin 43 (Huang R., et al., Cancer Res. 62: 2806-2812 (2002); Soroceanu L., et al., Glia 33: 107-117 (2001)), PKR (Shir A. and Levitzki A., Nat Biotechnol. 20: 895-900 (2002)), neurofibromin (NF1) (Cichowski K. and Jacks T., Cell 104: 593-604 (2001); Gutmann, D. H., et al., Hum. Mol. Genet. 10: 3009-3016 (2001)).

Growth factors and growth factor receptors

Several families of growth factors and related growth factor receptors including FGF-receptors and other important extracellular signaling molecules for cell differentiation and maintenance, like members of the BMP, TGF, IGF, TNF families are found to be impacted in more than one organ by treatment with FGF23CTP (Table 1).

Table 1 Genes for growth factors and receptors

		Treatment group of 2 males and 2 females	
		Control	FGF23CTP
Gene name	Organ	Gene expression level after 2 weeks (mean and range)	
Activin A receptor type II	ROHY	24.8 (20 to 32.6)	54.9 (37.2 to 78.7)
	Adrenal	20.6 (7.8 to 34.1)	58.5 (34.3 to 72.8)
	Retina	57.4 (48.2 to 70.6)	34.1 (27.7 to 38)
Bone morphogenetic protein 10	Kidney	74.6 (68.9 to 79.7)	138.6 (114.9 to 172.3)
	Liver	96.4 (92.3 to 103.3)	134.1 (108.8 to 152.6)
Bone morphogenetic protein 1	Adrenal	131.1 (81.5 to 150.6)	210.6 (147.5 to 361.8)
Growth differentiation factor 1	ROHY	866 (753.5 to 911.2)	1'410.2 (1'333.2 to 1'542.9)
Bone morphogenetic protein 2A	Duodenum	121.3 (100.4 to 146.9)	55.3 (37.9 to 80.1)
Bone morphogenetic protein receptor, type II	Adrenal	64.6 (57.9 to 69.8)	101.25 (71.2 to 156.5)
Colony-stimulating factor-1	ROHY	535.7 (470 to 598.2)	802.5 (667.6 to 884.5)
	Kidney	424.3 (316.1 to 576.8)	659.75 (574.5 to 870.1)
	B marrow	1'269.2 (934.5 to 1'493.8)	981.3 (782.2 to 1'091.5)
Epidermal growth factor receptor	ROHY	156.5 (138.4 to 167)	103.1 (88.4 to 112.1)
EGF-response factor 1	AV node	1'141.3 (1'044.4 to 1'317.9)	1'819.7(1'641.5 to 2'170.2)
EPHB2v protein-tyrosine kinase	ROHY	173.5 (119.8 to 204.5)	119.1 (113.2 to 121.8)
	Liver	52.85 (35.2 to 64.7)	82.6 (61.6 to 95.6)
FGF receptor-1	ROHY	669.9 (637 to 699.9)	932.4 (782.6 to 1'125.6)
	Thymus	224.5 (209.1 to 248.5)	153.2 (133.2 to 169.6)
	Adrenal	521.3(482.8 to 599)	624.2 (559.4 to 721.9)
Farnesol receptor HRR-1	Liver	267.7 (259.6 to 276.4)	383.3 (342.5 to 460.8)
	Duodenum	28.3 (21.3 to 36.4)	53.1 (44.6 to 67.1)
Insulin-like growth factor binding protein 2	AV node	1'371.2 (1'134 to 1'523.8)	880.75 (707.4 to 1'226.9)
	Liver	5'840.5 (4'269.2 to 6'849.6)	3'858.6 (3'160.1 to 5'301.8)
	Retina	1'396.8 (723.4 to 2'068.7)	807.9 (506.4 to 1'143.3)
Insulin-like growth factor I	Muscle	157.4 (115.9 to 179.9)	107.65 (83.5 to 140.1)
	Liver	649.675 (429.7 to 879.7)	1'055.4 (838.1 to 1'331.6)
Insulin-like growth factor binding protein 1	Liver	3'238.1 (2'346.3 to 3'807.7)	1'517 (105.7 to 2'449.4)
Insulin-like growth factor II	AV node	401.275 (359.7 to 462.2)	565.275 (524.7 to 619.9)
	Liver	426.5 (401.1 to 482.9)	606.9 (554 to 687.6)
	B Marrow	295.4 (251.2 to 339.8)	209.5 (183.5 to 236.8)
Neuregulin 1	Kidney	66.3 (42.7 to 105.8)	150.725 (119.4 to 193.9)
	Hippocampus	125.4 (105.1 to 133.3)	86.075 (70.7 to 97.8)
Retinoid X receptor-beta	ROHY	136.7 (120.7 to 154.4)	106.925 (84.4 to 139.5)
Retinoid X receptor-gamma	Muscle	253.925 (193.1 to 285.1)	580.9 (391.3 to 704.5)
Transforming growth factor β 2	Muscle	20.85 (20 to 23.4)	58.3 (39.3 to 66.9)
Transforming growth factor β 3	ROHY	37.9 (20 to 65.6)	88.4 (77 to 104.1)
Tumor necrosis factor receptor-2	ROHY	70.3 (43.2 to 92.2)	126.4 (117.8 to 136.4)

TNFR-related death receptor-6	Re atrium	100.6 (93.7 to 109.3)	144.65 (123.9 to 175.5)
	ROHY	155.1 (128.2 to 181.7)	98.8 (69.1 to 137.3)
TNFR-related death receptor-3	Muscle	119.5 (92.9 to 133.6)	82.4 (43.2 to 104.5)

ROHY: rostral hypothalamus; B Marrow: bone marrow; AV node: heart atrioventricular (AV) node.

Angiogenesis/Vasculogenesis

Transcript level changes for genes specifically involved in angiogenesis/vasculogenesis are found in several organs of animals treated with FGF23CTP (Table 2).

Table 2 Genes for angiogenesis

		Treatment group of 2 males and 2 females	
		Control	FGF23CTP
Gene name	Organ	Gene expression level after 2 weeks (mean and range)	
Vascular endothelial growth factor-A (VEGFA)	ROHY	491 (431.1 to 570.1)	326.175 (309.6 to 366.3)
	AV node	170.3 (106.6 to 234.9)	363.7 (302.7 to 506.7)
VEGFB	ROHY	484 (421.6 to 535.3)	626.2 (527.4 to 776.7)
Placental growth factor	Muscle	45.6 (20 to 78.4)	91.4 (60.8 to 112.6)
FLT receptor tyrosine kinase 1	Duodenum	30.1 (20 to 60.5)	70.7 (52.3 to 80.6)
	AV node	56.5 (22.7 to 76.1)	120.1 (77 to 156.4)
FLK1 receptor tyrosine kinase	Adrenal	57.1 (49 to 62.5)	42.7 (32.1 to 51)
Endothelial differentiation receptor 6	Muscle	258.6 (215.2 to 328)	181.9 (167.9 to 199.7)
	AV node	161.1 (111.6 to 188)	306.9 (251.2 to 365.4)
Endothelial differentiation receptor 4	Bone Marrow	261.3 (214.2 to 356.9)	494.8 (355.9 to 700.9)
	Liver	279 (227.2 to 361)	391.7 (347.7 to 453)
Brain-specific angiogenesis inhibitor 2	Retina	27.1 (20 to 38.2)	153.2 (82.2 to 201)
Brain-specific angiogenesis inhibitor 1 (MAS5)	ROHY	97.8 (82.8 to 118.8)	225.1 (169.2 to 283.8)
VE-Cadherin	Adrenal	63.8 (46.7 to 86.3)	110.2 (108 to 113.8)
Angiopoietin-1	Bone Marrow	198.6 (175.4 to 221.8)	123.2 (106.3 to 151.1)
	ROHY	235.7 (204.7 to 306.9)	162.5 (154.4 to 179.6)
TEK tyrosine kinase, angiopoietin receptor	Bone Marrow	91.8 (67.1 to 106.3)	52.7 (33.3 to 63.7)

ROHY: rostral hypothalamus; AV node: heart atrioventricular (AV) node.

Cycling: Retinoblastoma checkpoint

Transcript levels of genes involved in cell cycle control, especially those genes involved in the transition from the G1 to S-phase, are affected in several organs by treatment with FGF23CTP (Table 3). In particular, expression of those genes upstream or downstream of the retinoblastoma gene product (Rb) phosphorylation step, a major downstream control step for growth factor-induced proliferation, is altered. FGF23CTP also affects transcript levels for cyclin-dependent kinase 4 and cyclins D2, D3 and E2 involved in Rb phosphorylation. A second control level is also mobilized with cyclin kinase inhibitors like

p19INK4D, p21CIP1 and p27Kip1. Also affected is the inhibitor of p53 and Rb, Mdm2. The targets of the retinoblastoma protein are also involved: E2F1, E2F2, E2F5 and their binding partner Dp-2.

Table 3 Cell cycling

		Treatment group of 2 males and 2 females	
		Control	FGF23CTP
Gene name	Organ	Gene expression level after 2 weeks (mean and range)	
Cyclin-dependent kinase 4	ROHY	266 (240.1 to 283.7)	197.6 (167.6 to 231.7)
Cdk-inhibitor p57KIP2 (KIP2)	Duodenum	233.7 (212.1 to 251.9)	138.6 (114.9 to 172.3)
	Thymus	312.7 (292 to 341.1)	247.9 (227.3 to 284.2)
Cyclin A/CDK2-associated p19 (Skp1)	ROHY	1'349.8 (1'306.6 to 1'420.1)	1'069.6 (902.3 to 1'278)
Cyclin D2	Retina	210.2 (172.2 to 233.2)	132 (96.2 to 171.5)
	ROHY	231.8 (201.1 to 248.6)	197.1 (182.7 to 203.9)
Cyclin D3	Muscle	182.4 (157.2 to 198.2)	149.2 (135 to 162)
Cyclin-dependent kinase 5	Hippocampus	213.9 (172.3 to 254.7)	118.7 (96.6 to 162.3)
Cyclin E2	Bone Marrow	407 (367.4 to 491.4)	257 (175.2 to 294.8)
Cyclin I	Duodenum	1'109.9 (1'035.5 to 1'157.2)	900.6 (758.7 to 1'054.7)
E2F-1	Kidney	94 (51.9 to 150.9)	198.5 (135.8 to 263)
E2F-4	Muscle	888 (765.7 to 981.2)	643.8 (471.5 to 765.7)
	Hippocampus	208.7 (185.7 to 221.5)	153.4 (116.7 to 228.6)
	ROHY	812 (748.4 to 859.5)	245.9 (63.2 to 581.9)
	Kidney	505.6 (432.1 to 574.8)	741.6 (652.5 to 802.8)
E2F-5	Adrenal	53.9 (49.2 to 57.6)	39.6 (33.5 to 45)
E2F dimerization partner 2 (DP2)	Bone Marrow	2'091 (1'617.9 to 2'735.1)	1'222 (1'021.1 to 1'387.7)
Mdm2	Bone Marrow	40.925 (33.4 to 44.3)	20
Cdk-inhibitor p19	ROHY	163.6 (148.6 to 194.9)	267.8 (243.2 to 314.2)
Cdk-inhibitor 1A p21 (Cip1)	Adrenal	106.9 (85 to 125.1)	51.75 (20 to 96.8)
p53 binding protein	Muscle	269.35 (173 to 374.7)	138.7 (97.7 to 172)
	ROHY	217.5 (210.1 to 226.3)	154.7 (143.7 to 159.9)
Retinoblastoma 1	ROHY	255.9 (248.3 to 266.1)	467.9 (361.1 to 570)
Retinoblastoma binding protein 4	ROHY	361.1 (283.4 to 398.7)	164 (131.5 to 225.4)
S-phase response (cyclin-related)	ROHY	231.5 (199.8 to 270.3)	176 (159.8 to 205.6)
	Re atrium	126.75 (100.7 to 146.9)	159.1 (153.4 to 168.9)
RalGDS/AF-6 (Inhibitor of Cyclin D1)	ROHY	414.9 (390.1 to 439.7)	300.5 (284 to 313.9)
		438.1 (392.6 to 520.8)	354.5 (335.8 to 369.9)

ROHY: rostral hypothalamus.

Rostral hypothalamus

The rostral hypothalamus is the organ with the most pronounced changes in transcript levels in animals treated with FGF23CTP. Table 4 reflects genes of defined pathways and cellular actions with the most significant changes in this organ. In particular, effects on cytoarchitecture genes are especially pronounced in brain tissues.

Table 4 Rostral hypothalamus genes

	Treatment group of 2 males and 2 females	
	Control	FGF23CTP
Gene name	Gene expression level after 2 weeks (mean and range)	
Receptors		
Retinoic acid receptor	31.2 (26.8 to 41)	110 (100.9 to 122.8)
Cholecystokinin A receptor	87.1 (66.6 to 119.4)	196 (172.3 to 218.3)
Jagged 1	417.2 (402.1 to 437.7)	70.3 (20 to 161.9)
Protein kinases		
c-abl	214.4 (187.3 to 252.5)	119.4 (103.4 to 127)
c-yes	116.65 (107.9 to 127.8)	64.7 (50.8 to 77.4)
c-raf	404.7 (370.8 to 458.7)	327.5 (281.6 to 356.9)
Pim1	487.7 (432.9 to 557.6)	327.5 (281.6 to 356.9)
FYN	1'086.1 (1'018.4 to 1'172.4)	958.1 (891.3 to 1'007.6)
PKR Interferon-inducible	179.475 (162 to 192.7)	128.25 (112.1 to 140.9)
TYRO3	90.45 (78.7 to 106.8)	163.1 (134.7 to 179.5)
Axl	122.8 (112.4 to 140)	60.3 (34.7 to 89.4)
TrkB	108.6 (87.5 to 127.9)	40.8 (23.6 to 59.4)
Erk3	588.5 (551.5 to 679.4)	409.5 (370.5 to 482.7)
Phosphoinositide 3-kinase	90.3 (78.2 to 111.9)	241.5 (215.7 to 289.2)
Glycogen synthase kinase 3	70.4 (66.3 to 75.3)	155.5 (127.9 to 180.1)
JNK2	137.225 (122.2 to 159.8)	79.9 (64 to 95.2)
Ribosomal protein kinase B (RSK-B)	49.8 (35.4 to 74.5)	120 (90.4 to 141.8)
Janus kinase 1	106.7 (88.9 to 136.3)	43.3 (20 to 64.7)
SFRS protein kinase 2	396.1 (369.5 to 420.3)	205.8 (180.3 to 249.6)
Mnb (Minibrain)	567.4 (561.4 to 571.6)	446 (410.2 to 477.2)
Other signaling molecules		
Phosphoinositide-3-kinase, catalytic, delta polypeptide	90.3 (78.2 to 111.9)	241.5 (215.7 to 289.2)
RAS p21 protein activator	243.9 (211.5 to 270)	163 (139.4 to 198.5)
STAT1	182 (171.2 to 188.5)	119.3 (110.6 to 134.5)
Calcineurin A1	410.5 (367.6 to 486.4)	173.975 (114.5 to 239.7)
14-3-3 protein tau	2'110 (1'964.8 to 2'211.2)	1'748.5 (1'649.5 to 1'852)
Neurofibromin	78.6 (74.5 to 88.1)	144 (126.4 to 155.8)
Phosphodiesterase 4B	123 (110.1 to 133.3)	60.6 (46.7 to 72.3)
Phospholipase C	125.9 (107.4 to 136.2)	72.1 (56.8 to 81)
Transcription factors		
Hypoxia-inducible factor 1 alpha	611.5 (515.7 to 745.1)	286.5 (223 to 339.5).1)
CREB binding protein	165.4 (160 to 176.9)	93 (82.4 to 100)
Jun D	4'882.5 (4'592.6 to 5'361.6)	6'499.1 (6'353.9 to 6'745.1)
Inhibitor of DNA binding 4	140.3 (113.3 to 157.9)	67.6 (55.9 to 72.7)
Bmi-1 homeobox gene repressor	553.7 (513.6 to 599.8)	374.7 (352.6 to 410.4)
Solute transport		
Ca2+-ATPase	325.1 (274.6 to 401)	174.4 (130.8 to 247.5)
Sodium/hydrogen exchanger	1'062.4 (1'039.5 to 1'095.9)	785.5 (743.4 to 851.2)
Anion exchanger	627.1 (555.6 to 676.3)	844.5 (760.8 to 916.2)

Na,K-ATPase	2'032.2 (1'743.5 to 2'239.6)	1'453.2 (1'246.3 to 1'736.8)
CMP-sialic acid transporter	265.8 (241 to 329.6)	135.075 (112.8 to 159.1)
Vesicl transp rt		
RAB1	293.5 (246.9 to 320.3)	143.9 (93.8 to 214.9)
RAB5	1'266.2 (1'146.6 to 1'349)	809.8 (745.3 to 894)
RAB11A	554.8 (544.8 to 565.4)	344.9 (284.1 to 395.4)
RAB11B	182.3 (153.2 to 207.4)	71.2 (41.8 to 110.9)
RNP24	867.4 (809.5 to 903.7)	558.2 (486.3 to 642.9)
SNARE	140 (127.2 to 158.5)	55.4 (41.2 to 69.7)
GDP dissociation inhibitor beta	166.6 (156.5 to 173.4)	125.8 (104.1 to 142.6)
NIPSNAP2 (glioblastoma co-amplified)	212.9 (188.1 to 226.2)	118.7 (88.9 to 172)
Apoptosis		
Bcl-2-binding protein Nip3	1'099 (1'056.1 to 1'135.1)	794.8 (709.9 to 841.2)
BAX	30.95 (20 to 57.2)	134.4 (75.2 to 171)
Cytoarchitecture		
Tropomyosin 2 (beta)	20	131.6 (112.4 to 150.2)
Myosin regulatory light chain	328.5 (298.3 to 374)	177.2 (153.5 to 214)
Tropomyosin 4	72 (54.7 to 86.6)	140.6 (126.5 to 166.8)
Pinin, Desmosome associated protein	181.7 (164 to 203.2)	119.7 (108 to 131.5)
Connexin 43	410.4 (321.4 to 446.4)	184.5 (132.2 to 265.8)
Delta 2 catenin	239.6 (178.9 to 281.2)	118 (103.9 to 146.9)
Reticulon 4	2'343,7 (2'019.1 to 2'516.7)	1'309.2 (1'126.2 to 1'645.4)
Neural cell adhesion molecule 1	428 (297.1 to 524.2)	988.6 (841.6 to 1'196)
Amyloid beta precursor protein (cytoplasmic tail)-binding protein 2	400 (351.1 to 435)	191.3 (161.2 to 218.8)
Myelinization		
Oligodendrocyte myelin glycoprotein OMGP	1'144 (1'037.9 to 1'260.2)	773.5 (619.7 to 986)
Myelin oligodendrocyte glycoprotein NPD	176.8 (139.9 to 209.4)	62.7 (25.9 to 80.7)
Sphingomyelinase	585.5 (544.2 to 637.7)	259.5 (229.5 to 281.4)

Experiment 2

Methods

Seven days old C57/Bl6J mice are put together with their nursing parent in a sealed container ventilated by a mixture of oxygen and compressed air to a final oxygen concentration of 75% ± 2% until day 12. Returned at room air, the animals develop a relative ischemia of the retina with neovascularization starting as early as day 14.

On day 12, when animals returned from the oxygen incubation, a volume of 2.0 µl is injected in the eye using a glass pipette with a diameter of about 150 µm at its tip. The right eye is injected with 6 microgram (µg) FGF23CTP (3 µg / µl) and the left eye of the animal with PBS as individual control.

For the intravitreal injection of a substance at day 12, mice are anesthetized by inhalation of isoflurane. Five days later the animals are sacrificed after perfusion with dextran-fluorescein and retinal flatmounts are prepared. The coded whole mounts allow to evaluate the vascular changes of the retinal blindly. The proliferation score includes quantification of the proliferation including the papilla, vascular development, vasoconstriction, retinal bleeding and tortuosity of the vessels, features also seen in human retinal disease.

Animals

In total, 44 animals are used for injection with FGF23CTP and PBS. Of those, 8 animals died during hyperoxia or after injection, 2 animals are not perfused, 5 animals are paraffin embedded for evaluation of neurons, of which only three could be examined, and from 29 of these animals retinal flatmounts are prepared.

Evaluation of the angioproliferative changes

Evaluation of the angioproliferative changes in the retinal preparations is performed in a masked way on coded preparations. Retinopathy scoring system is adapted from Higgins, R. D., et al. (J. AAPOS 3: 114-116 (1999)) that was developed after modification of a scoring system used clinically in the neonatal intensive care unit. The following features are taken into consideration: neovascularisation of the optic disc, blood vessel tufts formation, large clusters of blood vessel tufts, central vasoconstriction and tortuosity of the vessels. Retinal haemorrhages are not taken into account as they may also result from the intraocular injection.

For each criteria, a defined number of points (P) is assigned, which are summed up to a total proliferation score. The higher the score, the worse the hypoxia induced retinopathy.

In detail:

Neovascularisation of the optic disc: either 1 or 0 points are assigned in case optic disc proliferation is measured (1 P) or absence (0 P).

Blood vessel tufts formation: the score for blood vessel tufts is determined by dividing every clock hour of the preparation surface in 4 zones: Zone 1 (optic disc zone) is evaluated in addition; Zone 2: one point for every wing (maximal 4 P); Zone 3: one point for every clock hour (maximal 12 P); and Zone 4: one point for every clock hour (maximal 12 P). Thus, a

maximum of 28 points is measured provided that no avascular areas exist, and a maximum of 12 points is calculated in case of complete avascularization.

Large clusters of blood vessel tufts: every large cluster of blood vessel tufts covering more than 3 sectors is counted as one point. Thus, maximal 8 points can be measured provided that no avascular areas exist, and maximal 4 points are assignable for complete avascularization.

Central vasoconstriction: 1 point is assigned to a vasoconstriction of zone 1 of greater than 50%; 1 point is allocated if the vasoconstriction is greater than 50% of every wing of zone 2 and 3. Thus, maximal 1 point can be assigned to zone 1, and maximal 4 points each for zones 2, 3 and 4. Since so far no vasoconstriction is ever observed for zone 4, a maximum of total 9 points (without zone 4) can be allotted.

Tortuosity of the vessels: 2 points are assigned if less than one-third of the vessels are tortuous; if the amount of tortuous blood vessels is between one-third and two-thirds, 4 points are allocated, and in case more than two-thirds of the vessels are tortuous, than 6 points are given.

The theoretical maximum of points is never reached, e.g. Zone 4 never shows avascular zones. Proliferation in avascular zones is never observed. This is in principle a mutual exclusion of criteria; the existence of vascular proliferations has a higher impact on the retinopathy score than large avascular areas. Therefore, the maximum score ever counted with this system is 38 points using a substance which is considered to enhance vascular proliferation.

The proliferation score relating to vascular changes on retinal flatmounts for individual animals treated with FGF23CTP (right eye) and PBS control (left eye) are measured as described above. The severity of retinopathy varies from animal to animal. Table 5 summarizes the determined proliferation score.

Table 5 Proliferation score

	right eye	left eye		right eye	left eye
Animal No.	FGF23CTP	PBS	Animal No.	FGF23CTP	PBS
146.1	4	4	150.1	14	16
146.2	13	8	150.2	8	26
146.3	8	9	150.3	17	24
146.4	3	5	150.4	30	29
146.5	6	8	160.2	10	7

146.6	1	9
146.7	2	4
146.8	5	6
147.1	9	8
147.2	8	9
147.3	2	8
147.4	11	12
147.5	1	11
149.1	5	26
149.2	14	36
149.3	8	10
149.4	17	12
149.5	10	8
160.3	10	7
160.4	16	20
160.5	28	28
161.1	30	15
161.2	21	33
161.3	17	22

The proliferation scores of all animals are further displayed as box plot in Figure 1. Comparing the individual treated (right eye, FGF23CTP) with control eye (left, PBS)) the effect of FGF23CTP injection reaches statistical significance in a paired t-test with p= 0.04 (Table 6).

Table 6 Paired t-test

Mean Difference	DF	t-Value	P-Value
-3,172	28	-2,183	0,0375

The proliferation scores for the separate factors are presented in Table 7, and the respective paired t-tests in Tables 8 to 10.

Table 7 Proliferation scores

proliferation sum		blood vessel tufts		large clusters of prolif.		avascular zone		tortuosity of vessels	
right eye	left eye	right eye	left eye	right eye	left eye	right eye	left eye	right eye	left eye
FGF23CTP	PBS	FGF23CTP	PBS	FGF23CTP	PBS	FGF23CTP	PBS	FGF23CTP	PBS
2	2	2	2	0	0	1	2	1	0
10	8	10	8	0	0	3	0	0	0
7	9	6	9	0	0	1	0	0	0
2	4	2	4	0	0	1	1	0	0
4	8	4	8	0	0	2	0	0	0
1	9	1	9	0	0	0	0	0	0
1	2	1	2	1	1	1	1	0	1
4	6	3	5	1	0	1	0	0	0
6	4	6	4	0	0	1	1	2	3

5	6	5	6	0	0	0	0	3	3
0	4	0	4	1	1	1	1	1	3
7	7	7	7	2	2	2	2	2	3
0	7	0	7	0	0	0	0	1	4
2	17	2	14	0	2	3	4	0	5
8	25	7	20	1	5	4	6	2	5
4	6	4	5	0	1	3	3	1	1
10	6	10	6	0	0	2	2	5	4
6	4	6	3	0	0	3	3	1	1
8	9	7	7	1	2	4	5	2	2
4	21	4	17	0	4	3	4	1	4
11	14	10	13	0	1	4	6	2	4
17	18	14	15	3	3	7	5	6	6
6	3	5	3	1	0	3	3	1	1
6	5	6	5	0	0	2	2	2	0
12	14	7	11	4	3	3	4	1	2
18	20	14	16	3	4	6	6	4	2
18	9	13	7	4	2	6	4	6	2
14	21	11	15	5	3	5	7	2	5
12	16	9	11	2	5	4	5	1	1

Table 8 Paired t-test proliferation sum

Mean Difference	DF	t-Value	P-Value
-2,724	28	-2,514	0,0180

Table 9 Paired t-test avascular zone

Mean Difference	DF	t-Value	P-Value
-0,034	28	-0,154	0,8791

Table 10 Paired t-test tortuosity of the vessels

Mean Difference	DF	t-Value	P-Value
-0,517	28	-1,543	0,1341

Evaluation of the toxicity

From the five mice originally planed for evaluation of neuronal damage after injection of FGF23CTP in the right eye and PBS in the left eye, only three are studied due to damage to the lens in one eye and fixation problems in another animal.

The total thickness of the outer nuclear layer, the inner nuclear layer and the ganglion cell layer is measured using a standardized magnification after paraffin embedding and hematoxylin and eosin (HE)-staining. Three sections of each eye are measured at three locations each. Table 11 shows the result of the measurements in mm using a x200 magnification. Section are encoded for evaluation.

Table 11 Neuronal Damage

Animal No.	Outer nuclear layer		Inner nuclear layer		Ganglion cell layer	
	right	left	right	left	right	left
150-5	10,5	11	6	6,5	2,5	2
	11	9	7	5	3	2
	10,5	10	5	5,5	2	3
	10	10	5,5	5	2	1,5
	10	10,5	7	6	2,5	3,5
	11	10	6	7	2	2
	11	9	6,5	5	2,5	1,5
	11,5	11	6,5	7	2,5	2,5
	10	10	6,5	6	2	2
150-7	10,5	10,5	6	6	1,5	2
	12	12	7	7	2	2
	10	9	6	5,5	2,5	3
	10	11	6	7	2	2
	10	11	6	6	2	2,5
	11,5	11	7	7	2,5	3
	10	11	6	6,5	2	2
	10	12,5	5,5	6	2	2
		10		7		2,5
150-9	10,5	10	5	6	2	2
	11	10	6	6	2	2,5
	10,5	11	6	5,5	2	1,5
	11	9	5	6	2	2,5
	11	11	5	5	2,5	2
	12	11	6	5,5	2,5	2,5
	10	12	5,5	6	3	2

	11	9	5,5	6	3	2,5
	10	11	6,5	5	2	1,5
variance	0,64121279	0,9600273	0,63245553	0,69337525	0,38078866	0,50636968
average	10,6346154	10,462963	6	6	2,25	2,22222222

Based on the measurements performed on the three animals, FGF23CTP is found not to induce any neuronal damage in the eye. In detail, there is no marked difference in the thickness of the neuronal layers of the inner nuclear layer, the outer nuclear layer or the ganglion cell layer five days after the injection of either FGF23CTP or PBS.

Claims

1. Use of a polypeptide for the manufacture of a medicament for use in the treatment of a disease associated with deregulated angiogenesis wherein the polypeptide is selected from the groups consisting of

a) fibroblast growth factor 23 (FGF-23) (SEQ. ID No: 1) or a fragment of FGF-23;

b) a bioactive polypeptide having a percentage of identity of at least 50% with the amino acid sequence of any one of the polypeptides of (a);

c) a bioactive variant of any one of the polypeptides of (a) or (b).

2. Use of a polypeptide according to claim 1, wherein the disease associated with deregulated angiogenesis is selected from the group of retinopathies, age-related macular degeneration, haemangioblastoma, haemangioma and tumors.

3. Use of a polypeptide according to claim 1 or 2, wherein the disease associated with deregulated angiogenesis is retinopathy.

4. Use of a polypeptide as defined in any one of the groups (a), (b) or (c) of claim 1 for the manufacture of a medicament for use in the treatment of a cell proliferative disorder.

5. Use of a polypeptide according to claim 4, wherein the cell proliferative disorder is selected from the group of chronic or acute renal diseases, arteriosclerosis, atherosclerosis, psoriasis, endometriosis, diabetes, chronic asthma and cancer.

6. Use of a polypeptide according to claim 4 or 5, wherein the cell proliferative disorder is cancer.

7. Use of a polypeptide according to any one of claims 1 to 6, wherein the polypeptide is encoded by a nucleic acid which hybridizes under stringent conditions to SEQ. ID No: 3.

8. Use of a polypeptide according to any one of claims 1 to 7, wherein the polypeptide comprises a C-terminal fragment of FGF-23.

9. Use of a polypeptide according to claim 8, wherein the polypeptide comprises at least 15 amino acids of the C-terminus of FGF-23.

10. Use of a polypeptide according to claim 8 or 9, wherein the polypeptide has an amino acid sequence of SEQ ID No: 2.

11. Use of a polypeptide according to any one of claims 8 to 10, wherein the polypeptide is encoded by a nucleic acid which hybridizes under stringent conditions to SEQ. ID No: 4.

12. A method for the treatment of a disease associated with deregulated angiogenesis comprising administering an effective amount of a polypeptide to a mammal including a human suffering from the disease, wherein the polypeptide is selected from the groups consisting of

a) fibroblast growth factor 23 (FGF-23) (SEQ. ID No: 1) or a fragment of FGF- 23;

b) a bioactive polypeptide having a percentage of identity of at least 50% with the amino acid sequence of any one of the polypeptides of (a);

c) bioactive variant of any one of the polypeptides of (a) or (b).

13. A method according to claim 12, wherein the disease associated with deregulated angiogenesis is selected from the group of retinopathies, age-related macular degeneration, haemangioblastoma, haemangioma and tumors.

14. A method according to claim 12 or 13, wherein the disease associated with deregulated angiogenesis is retinopathy.

15. A method for the treatment of a cell proliferative disorder comprising administering an effective amount of a polypeptide as defined in any one of the groups (a), (b) or (c) of claim 12 to a mammal including a human suffering from the disorder.

16. A method according to claim 15, wherein the cell proliferative disorder is selected from the group of chronic or acute renal diseases, arteriosclerosis, atherosclerosis, psoriasis, endometriosis, diabetes, chronic asthma and cancer.

17. A method according to claim 15 or 16, wherein the cell proliferative disorder is cancer.

18. The method for the treatment of a disease or disorder according to any of claims 12 to 17 in which the effective amount of the polypeptide is administered intravenously, intramuscularly, subcutaneously, orally or topically.

19. The method for the treatment of a disease or disorder according to any one of claims 12 to 18, wherein the polypeptide is encoded by a nucleic acid which hybridizes under stringent conditions to SEQ. ID No: 3.

20. The method for the treatment of a disease or disorder according to any of claims 12 to 19, wherein the polypeptide comprises a C-terminal fragment of FGF-23.

21. The method for the treatment of a disease or disorder according to claim 20, wherein the polypeptide comprises at least 15 amino acids of the C-terminus of FGF-23.

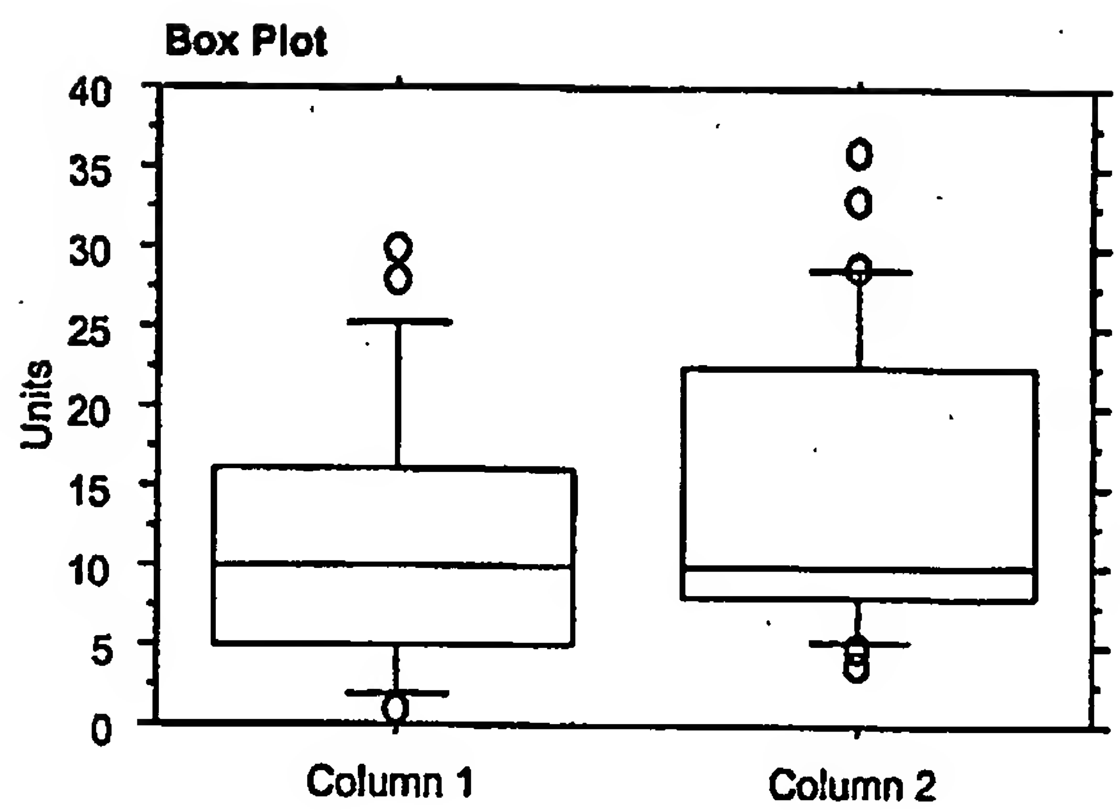
22. The method for the treatment of a disease or disorder according to claims 20 or 21, wherein the polypeptide has an amino acid sequence of SEQ ID No: 2.

23. The method for the treatment of a disease or disorder according to any one of claims 20 to 22, wherein the polypeptide is encoded by a nucleic acid which hybridizes under stringent conditions to SEQ. ID No: 4.

24. A pharmaceutical composition for use in a disease associated with deregulated angiogenesis comprising a polypeptide as defined in claims 1, and 7 to 11 and a pharmaceutically-acceptable carrier.

25. A pharmaceutical composition for use in a cell proliferative disorder comprising a polypeptide as defined in claims 1, and 7 to 11 and a pharmaceutically-acceptable carrier.

Figure 1 / 1



Column 1: right eye (FGF23CTP); Column 2: left eye (PBS), Units: proliferation score.

SEQUENCE LISTING

<110> Bollekens, Jacques

Chibout, Salah-Dine

<120> Use of organic compounds

<130> 4-33264P1

<160> 4

<170> PatentIn version 3.1

<210> 1

<211> 251

<212> PRT

<213> Homo sapiens

<400> 1

```
Met Leu Gly Ala Arg Leu Arg Leu Trp Val Cys Ala Leu Cys Ser Val
1               5                   10                  15

Cys Ser Met Ser Val Leu Arg Ala Tyr Pro Asn Ala Ser Pro Leu Leu
            20                  25                  30

Gly Ser Ser Trp Gly Gly Leu Ile His Leu Tyr Thr Ala Thr Ala Arg
        35                  40                  45

Asn Ser Tyr His Leu Gln Ile His Lys Asn Gly His Val Asp Gly Ala
    50                  55                  60

Pro His Gln Thr Ile Tyr Ser Ala Leu Met Ile Arg Ser Glu Asp Ala
65                  70                  75                  80
```

Gly Phe Val Val Ile Thr Gly Val Met Ser Arg Arg Tyr Leu Cys Met
85 90 95

Asp Phe Arg Gly Asn Ile Phe Gly Ser His Tyr Phe Asp Pro Glu Asn
100 105 110

Cys Arg Phe Gln His Gln Thr Leu Glu Asn Gly Tyr Asp Val Tyr His
115 120 125

Ser Pro Gln Tyr His Phe Leu Val Ser Leu Gly Arg Ala Lys Arg Ala
130 135 140

Phe Leu Pro Gly Met Asn Pro Pro Pro Tyr Ser Gln Phe Leu Ser Arg
145 150 155 160

Arg Asn Glu Ile Pro Leu Ile His Phe Asn Thr Pro Ile Pro Arg Arg
165 170 175

His Thr Arg Ser Ala Glu Asp Asp Ser Glu Arg Asp Pro Leu Asn Val
180 185 190

Leu Lys Pro Arg Ala Arg Met Thr Pro Ala Pro Ala Ser Cys Ser Gln
195 200 205

Glu Leu Pro Ser Ala Glu Asp Asn Ser Pro Met Ala Ser Asp Pro Leu
210 215 220

Gly Val Val Arg Gly Gly Arg Val Asn Thr His Ala Gly Gly Thr Gly
225 230 235 240

Pro Glu Gly Cys Arg Pro Phe Ala Lys Phe Ile
245 250

<210> 2

<211> 75

<212> PRT

<213> Homo sapiens

<400> 2

His Thr Arg Ser Ala Glu Asp Asp Ser Glu Arg Asp Pro Leu Asn Val
1   5   10   15

Leu Lys Pro Arg Ala Arg Met Thr Pro Ala Pro Ala Ser Cys Ser Gln
            20   25   30

Glu Leu Pro Ser Ala Glu Asp Asn Ser Pro Met Ala Ser Asp Pro Leu
        35   40   45

Gly Val Val Arg Gly Gly Arg Val Asn Thr His Ala Gly Gly Thr Gly
    50   55   60

Pro Glu Gly Cys Arg Pro Phe Ala Lys Phe Ile
65   70   75

<210> 3

<211> 756

<212> DNA

<213> Homo sapiens

<400> 3
atgttggggg cccgcctcag gctctgggtc tgtgccttgt gcagcgtctg cagcatgagc 60

gtcctcagag cctatcccaa tgcctcccca ctgctcggct ccagctgggg tggcctgatc 120

cacctgtaca cagccacagc caggaacagc taccacctgc agatccacaa gaatggccat 180

gtggatggcg caccccatca gaccatctac agtgccctga tgatcagatc agaggatgct 240

ggctttgtgg tgattacagg tgtgatgagc agaagatacc tctgcatgga tttcagaggc 300

aacatttttg gatcacacta tttcgacccg gagaactgca ggttccaaca ccagacgctg 360

gaaaacgggt acgacgtcta ccactctcct cagtatcact tcctggtcag tctgggccgg 420

gcgaagagag ccttcctgcc aggcatgaac ccacccccgt actcccagtt cctgtcccgg 480

aggaacgaga tcccccctaat tcacttcaac acccccatac cacggcggca cacccggagc 540

gccgaggacg actcggagcg ggacccccctg aacgtgctga agccccgggc ccggatgacc 600

ccggccccgg cctcctgttc acaggagctc ccgagcgccg aggacaacag cccgatggcc 660

agtgacccat taggggtggt caggggcggt cgagtgaaca cgcacgctgg gggaacgggc 720

ccggaaggct gccgcccctt cgccaagttc atctag 756

<210> 4

<211> 228

<212> DNA

<213> Homo sapiens

<400> 4

```
cacacccgga gcgccgagga cgactcggag cgggaccccc tgaacgtgct gaagccccgg      60
gcccggatga ccccggcccc ggcctcctgt tcacaggagc tcccgagcgc cgaggacaac     120
agcccgatgg ccagtgaccc attaggggtg gtcaggggcg gtcgagtgaa cacgcacgct     180
gggggaacgg gcccggaagg ctgccgcccc ttcgccaagt tcatctag                  228
```

INVENTOR INFORMATION

Inventor One Given Name:: Jacques
Family Name:: Bollekens
Postal Address Line One:: Hardstrasse 28
City:: Basel
Country:: Switzerland
Postal or Zip Code:: 4052
City of Residence:: Basel
Country of Residence:: Switzerland
Citizenship Country:: Belgium
Inventor Two Given Name:: Salah-Dine
Family Name:: Chibout
Postal Address Line One:: 5, route de Mulhouse
City:: Tagolsheim
Country:: France
Postal or Zip Code:: 68720
City of Residence:: Tagolsheim
Country of Residence:: France
Citizenship Country:: France

CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 001095

APPLICATION INFORMATION

Title Line One:: USE OF ORGANIC COMPOUNDS
Total Drawing Sheets:: 1
Formal Drawings?:: No
Application Type:: Provisional
Docket Number:: 4-33264P1
Secrecy Order in Parent Appl.?:: No

REPRESENTATIVE INFORMATION

Representative Customer Number:: 1095

Source:: PrintEFS Version 2.0